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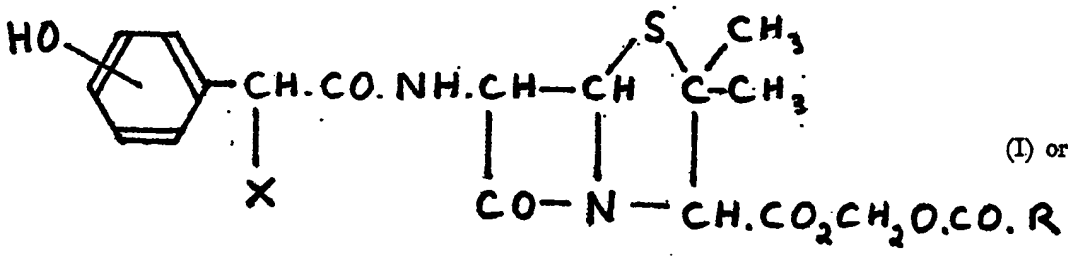
(54) DERIVATIVES OF α-AMINOPENICILLINS

(71) We, BEECHAM GROUP LIMITED, a British Company, of Beecham House, Great West Road, Brentford, Middlesex, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

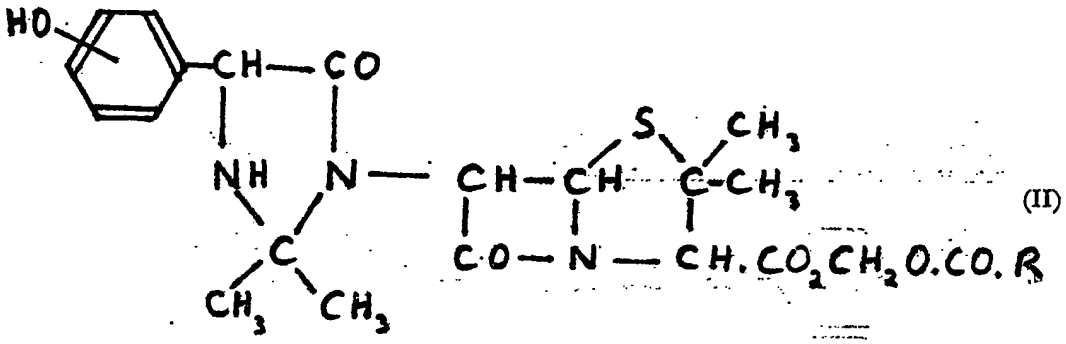
This invention relates to derivatives of α-aminopenicillins and is particularly concerned with acyloxymethyl esters of certain α-aminopenicillins and of the condensation products of such α-aminopenicillins with ketones. Such products are of value as antibacterial agents, as nutritional supplements in animal foods, as agents for the treatment of mastitis in cattle and as therapeutic agents in poultry and animals, including man, in the treatment especially of infectious diseases caused by Gram-positive and Gram-negative bacteria.

Whilst the compounds of the present invention exhibit the same broad-spectrum antibacterial activity as the α-aminopenicillins from which they are derived, they have an advantage in that they are well absorbed by the oral route in animals and man, and after absorption hydrolyse to give high blood levels of the appropriate α-aminopenicillins.

According to the present invention there is provided compounds of the general formula:—



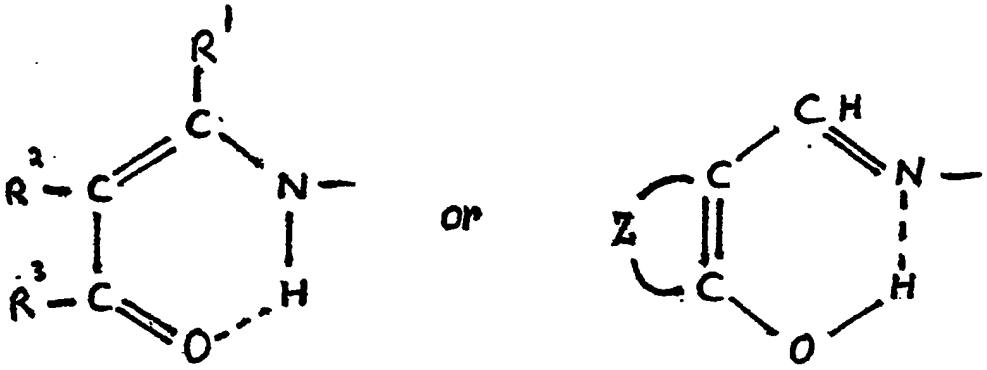
(I) or



(II)

[F

where R is an alkyl, cycloalkyl, alkenyl, cycloalkenyl, aralkyl, aryl or heterocyclic group any of which may be substituted and where in (I) X is NH_2, optionally in the form of an acid addition salt or a protected amino group of the general formula:—



(III) (IV)

or tautomeric modifications thereof, wherein the dotted lines represent hydrogen bonds, R^1 is a lower alkyl group, R^2 is either H or together with R^1 completes a carbocyclic ring, R^3 is a lower alkyl or lower alkoxy group, and Z represents the residue of a substituted or unsubstituted benzene or naphthalene ring.

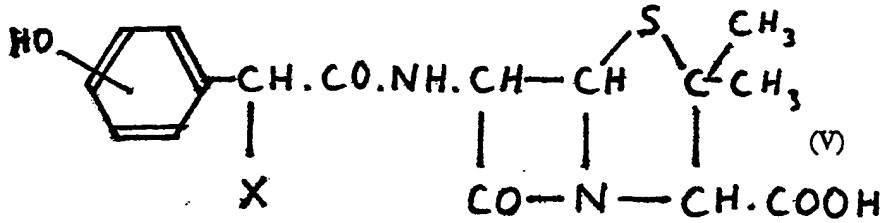
In the present specification the term "lower alkyl" and "lower alkoxy" refer to groups having from 1 to 6 carbon atoms.

In one of its preferred embodiments the present invention provides compounds of formulae (I) and (II) above wherein the group R is methyl or tertiary butyl and X is defined as above e.g. X is NH_2 or 1-methoxycarbonylpropyl-2-yl. These compounds i.e. the acetoxymethyl and pivaloyloxymethyl esters are particularly valuable in that the oral absorption characteristics are very satisfactory and high blood levels are obtained. Particular compounds of the present invention include pivaloyloxymethyl and acetoxymethyl 6-[(—)α-amino-α-(f-hydroxyphenyl)acetamido penicillanate and pivaloyloxymethyl and acetoxymethyl 6-[2,2-dimethyl-5-oxo-4-(f-hydroxyphenyl)-1-imidazolidinyl]penicillanate and non-toxic acid addition salts thereof.

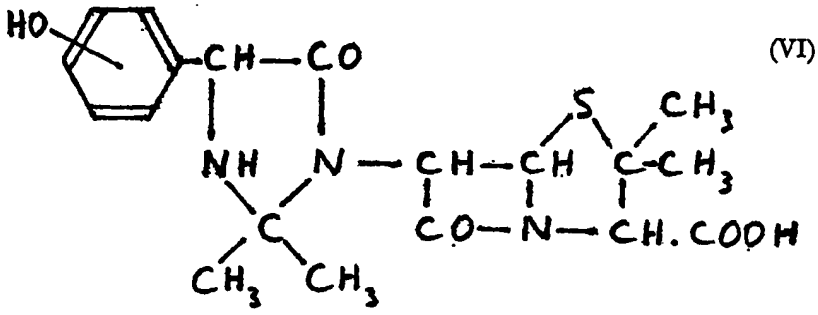
The present invention comprehends all those compounds of formula (I) and formula (II) irrespective of the location of the hydroxyl group in the benzene ring. Preferably, however, the hydroxyl group occupies the *p*-position on the benzene ring. The compounds can exist in epimeric forms, and the preferred epimers are those in which the side chain is derived from (—) α-amino-*p*-hydroxyphenylacetic acid.

In the compounds of this invention of formulae (I) and (II), the group R is alkyl, cycloalkyl, alkenyl, cycloalkenyl, aralkyl, aryl or heterocyclic. Examples of suitable alkyl groups include straight or branched chain lower alkyl radicals having from 1 to 6 carbon atoms such as methyl, ethyl, isopropyl, *n*-butyl, *sec*-butyl or *tert*-butyl, pentyl or hexyl. Suitable cycloalkyl groups include cyclopentyl, cyclohexyl, 1-adamantyl, cycloheptyl and cyclohexylmethyl while suitable cycloalkenyl groups include cyclopentenyl and cyclohexenyl. Examples of suitable aralkyl groups include benzyl, phenylethyl and phenylpropyl and suitable aryl groups include phenyl or substituted phenyl, 1- or 2-naphthyl or substituted naphthyl. Suitable heterocyclic radicals include pyridyl, pyrazinyl, pyrimidinyl, thienyl, furyl or isoxazolyl.

One method of preparing the esters of the present invention is to treat a salt (e.g. the Na or K salt) of the appropriate penicillanic acid (V) or (VI) with the appropriate acyloxymethyl bromide or chloride (VII: Y=Br or Cl) in an organic solvent such as acetone or dimethylformamide:—



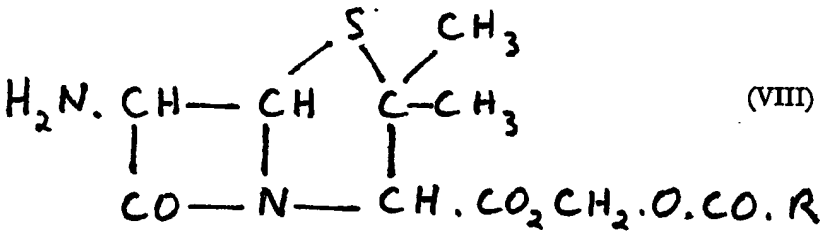
(V)



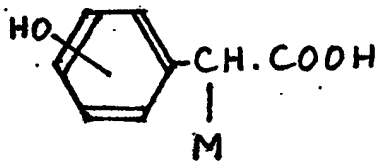
(VI)

$$Y.CH_2O.CO.R \qquad \text{(VII)}$$

An alternative way of making esters of general formula (I) it to treat the appropriate acyloxymethyl 6-aminopenicillanate (VIII) with a carboxyl activated derivative of a carboxylic acid of the general formula (IX) wherein M represents an amino group, a protected amino group or a group which may subsequently be converted into an amino group.



(VIII)



(IX)

If desired the phenolic hydroxy group may also be protected during the acylation step, *e.g.* as an O-benzyl or O-benzyloxycarbonyl group which is subsequently removed, but generally the protection of this group is not essential.

Examples of protected amino groups include the protonated amino group ($M=NH_3^+$) which after the coupling reaction reverts to NH_2 on simple neutralisation, the benzyloxycarbonylamino group ($M=NH.CO_2.CH_2.Ph$) or substituted benzyloxycarbonylamino groups which are subsequently converted to NH_2 by catalytic hydrogenation, and various groups which after coupling regenerate the amino group on mild acid or alkaline hydrolysis.

Examples of the group M which may subsequently be converted into NH_2 by mild acid hydrolysis include enamine groups of general formula (III) and (IV) above.

Examples of the group M which may subsequently be converted into NH_2 by mild alkaline hydrolysis include 2-sulphonyl substituted ethoxycarbonylamino groups $R.SO_2.CH_2.CH_2.O.CO.NH—$, wherein R represents a substituted or unsubstituted alkyl, aralkyl, or aryl group.

Another example of a group M which can be converted into NH_2 after coupling of the acid (II) with 6-aminopenicillanic acid is the azido group. In this case the final conversion into NH_2 may be brought about either by catalytic hydrogenation or by electrolytic reduction.

In carrying out the coupling of the acid (IX) to the penicillanate (VIII) the choice of activating group for the carboxyl function will be influenced by the chemical nature of the α-substituent M. Thus, when M is an acid-stable group such as the protonated amino group NH_3^+ or the azido group, it is often convenient to convert

the acid (II) into an acid halide, for example by treating it with thionyl chloride of phosphorus pentachloride to give the acid chloride.

Such reagents would however, be avoided when M is an acid-labile group of type (III) or (IV), in which case it is often convenient to make use of a mixed anhydride. For this purpose particularly convenient mixed anhydrides are the alkoxyformic anhydrides, which are conveniently prepared by treating an alkali metal or tertiary amine salt of the acid (II) with the appropriate alkyl chloroformate in an anhydrous medium at or below room temperature. Other ways of activating the carboxyl group include reaction with a carbodiimide to give a reactive O-acyl isourea or reaction with carbonyldiimidazole to give a reactive imidazolide. These latter derivatives, like the mixed anhydrides, are relatively unstable substances and hence are not usually isolated, the reaction with 6-aminopenicillanic acid being carried out *in situ*.

Another reactive derivative of α-amino-*p*-hydroxyphenylacetic acid useful in the preparation of the compound of the present invention is the Leuch's anhydride. In this structure the group which activates the carboxyl group also serves to protect the amino group.

Esters of general formula (I) where $X=NH_2$ may be converted into the corresponding imidazolidinone (II) by reaction with acetone, preferably present in excess, in neutral or basic conditions (e.g. (I), $X=NH_2$ may be set aside overnight in acetone containing triethylamine as a catalyst). The reaction with acetone is reversible so in the bloodstream (II) will loose acetone and generate (V), $X=NH_2$ via either (VI) or (I), $X=NH_2$.

The following Examples illustrate the invention:—

EXAMPLE 1

Acetoxymethyl 6-[(−)N-(1-methoxycarbonylpropen-2-yl) α-amino-α (p-hydroxyphenyl)acetamido]penicillanate.

(a) Resolution of α-benzyloxycarbonylamino-α-(p-hydroxyphenyl)acetic acid

A solution of the title acid (224 g) and quinine trihydrate (285 g) in boiling ethanol (2.5 l.) was allowed to cool. The crystals which separated were collected and recrystallised twice from ethanol to give a 76% yield of the quinine salt of the laevorotatory acid $[\alpha]_D^{20}-158.5°$ (C, 1 in MeOH). Treatment of the quinine salt (68 g) with dilute sodium hydroxide, removal of quinine by ether-extraction, and acidification of the aqueous solution gave the laevorotatory acid. This was crystallised twice from aqueous ethanol to give 28 g. (85%) of (−) α-benzyloxycarbonylamino-α-(*p*-hydroxyphenyl) acetic acid m.p. 159—161° C, $[\alpha]_D^{18}-120.0°$ (C, 1 in MeOH). (Found: C, 64.1; H, 5.2; N, 4.7. $C_{24}H_{21}NO_7$ requires C, 63.9; H, 4.9; N, 4.7%).

The mother liquor from the crude quinine salt was evaporated to dryness *in vacuo* to leave a syrup which was treated with aqueous sodium hydroxide and the quinine removed by ether-extraction. Acidification of the aqueous layer gave the crude dextrorotatory acid (108 g.) which was collected, dried, and treated with ephedrine (63 g.) in boiling ethanol (450 ml.). On cooling the solution the ephedrine salt of the dextrorotatory acid separated, and was collected and recrystallised from ethanol. Yield 111 g., $[\alpha]_D^{21}+46.8°$ (C, 1 in H_2O). The acid was recovered from this salt in the usual way to give (+)α-benzyloxycarbonylamino-*p*-hydroxyphenylacetic acid (96%) which, after recrystallisation from 50% aqueous ethanol, had m.p. 158—161° C, $[\alpha]_D^{21}$ +120.2° (C, 1 in MeOH). (Found: C, 64.1; H, 5.3; N, 4.8. $C_{24}H_{21}NO_7$ requires C, 63.9; H, 4.9; N, 4.7%).

(b) Preparation of (−)α-amino-α-(p-hydroxyphenyl)acetic acid

A suspension of (−)-α-benzyloxycarbonylamino-α-(p-hydroxyphenyl) acetic acid (21.8 g.) in water (180 ml.) was treated with dilute sodium hydroxide solution to give a clear solution of pH 8.7. 5% Palladium on calcium carbonate catalyst (2.2 g.) was added, and the mixture was shaken in hydrogen at atmospheric pressure until no more gas was absorbed. The catalyst was filtered off through a pad of kieselguhr and washed with water. The combined filtrate and washings were adjusted to pH 5.0 and the solution was evaporated *in vacuo* to small bulk. The crude product separated as a gelatinous precipitate which redissolved when the mixture was boiled. Colourless, crystalline (−)-α-amino-α-(p-hydroxyphenyl)acetic acid separated when the solution was cooled. It was collected, washed with a little cold water, and dried *in vacuo* over phosphorous pentoxide. The yield was 10.1 g. (83%), m.p. 225—226° $[\alpha]_D^{22}-108°$ (*c*, 1 in H_2O). (Found: C, 57.2; H, 5.4; N, 8.3%. $C_8H_9NO_3$ requires: C, 57.5; H, 5.4; N, 8.4%). N.M.R. (D_2O+NaOD): multiplet centred on 3.15 (4H, aromatic), singlet 5.8 (1H N—CH).

(c) Preparation of sodium (−)-N-(1-methoxycarbonylpropen-2-yl)-α-amino-α-(p-hydroxyphenyl)acetate

Methanolic sodium hydroxide (60 ml. 0.324N, 0.0195 mole) was added to (−)-α-amino-α-(p-hydroxyphenyl) acetic acid (3.4g. 0.02 mole) and washed in with methanol (20 ml.). When the suspension was heated almost to boiling a clear solution was obtained, but at reflux the crystalline sodium salt of the amino-acid separated. To the stirred, boiling suspension was added during 10 minutes methyl acetoacetate (2.4 ml., 0.022 mole) in methanol (20 ml.). After a further 20 minutes boiling a clear solution resulted and this was boiled for 20 minutes longer. The methanol was distilled off (bath temperature 130°) and replaced simultaneously at the same rate with dry toluene. When 90 ml. toluene had been added a white crystalline solid separated. The process was continued until the distillation temperature reached 100° and 170° ml. toluene had been added. The suspension was kept overnight at 5°, then the product was collected, washed with dry toluene, and dried *in vacuo* at 40° over phosphorus pentoxide. The yield of sodium (−)-N-(1-methoxycarbonylpropen-2-yl)-α-amino-α-(p-hydroxyphenyl)acetate was 4.5 g. (95%) Found:. C, 54.35; H, 4.9; N, 4.9%. $C_{18}H_{14}NO_5$ Na requires C, 54.4; H, 4.9; H, 4.9%). I.R. (Nujol): 3,300cm^{-1} (N—H—), 1655cm^{-1} (C=O—), 1560cm^{-1} (COO$^-$).

(d) Preparation of potassium 6-aminopenicillanate

A solution was prepared from 6-aminopenicillanic acid (4.32 g., 0.02 mole), water (50 ml.), and sufficient potassium hydroxide solution to bring the whole to pH 7.8. Acetone (80 ml.) was added, and the mixture cooled to −10°.

(e) Preparation of potassium 6-[(−)-N-(1-methoxycarbonylpropen-2-yl)-α-amino-α-(p-hydroxyphenyl)acetamido]penicillanate

Dry acetone (50 ml.) was stirred and held at −10° to −8° whilst ethyl chloroformate (2.0 ml., 0.021 mole) was added, followed by a 1% solution of N-methylmorpholine in acetone (3 ml.). Sodium (−)N-(1-methoxycarbonylpropen-2-yl)-α-amino-α-(p-hydroxyphenyl)acetate (5.74 g., 0.02 mole, prepared as described under (c), was added and washed in with ice-cold dry acetone (20 ml.). The mixture was stirred at −10° for 30 min. cooled to −25°, and filtered through kieselguhr. The filtrate was added to the stirred cold (−10°) solution of potassium 6-aminopenicillanate prepared as described under (d). The clear solution was stirred without external cooling for 30 min. and then evaporated to dryness under reduced pressure at a temperature below 20°. The residue was dried *in vacuo* over P_2O_5, then stirred with dry methanol (70 ml.) and filtered (charcoal and kieselguhr) to remove a little solid. The filtrate was treated with isopropanol (250 ml.) and cooled to 5°, whereupon colourless crystals of potassium 6-(−)-N-(1-methoxycarbonylpropen-2-yl)-α-amino-α-(*p*-hydroxyphenyl) acetamido]penicillanate separated. This product was collected washed with dry ether, and dried *in vacuo*. Yield 3.5 g. (Found: C, 49.3; H, 5.2; K, 7.6; N, 8.3; S, 6.4. $C_{21}H_{24}KN_3O_7S$, 1/2 H_2O requires C, 49.4; H, 4.9; K, 7.7; N, 8.3; S, 6.3%).

(f) Preparation of acetoxymethyl 6-[(−)N-(1-methoxycarbonylpropen-2-yl)α-amino-α(p-hydroxyphenyl)acetamido]penicillanate

The potassium salt, prepared as described under (e) above, mixed with dry acetone (20 ml.) was treated with the equivalent quantity (1.05 g.) of bromomethyl acetate and stirred at room temperature for 1 hour. The mixture was then filtered through kieselguhr to remove potassium bromide and the filtrate was poured into ice-water (120 ml.). The amorphous product which separated was collected, washed with cold water and dried over P_2O_5 *in vacuo* to give the required acetoxymethyl ester.

EXAMPLE 2

Acetoxymethyl 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]penicillanate

Acetoxymethyl 6-[(−)N-(1-methoxycarbonylpropen-2-yl) α-amino-α-(*p*-hydroxyphenyl)acetamido]penicillanate (5.35 g. 0.01 mole), prepared as described under Example I (f), mixed with water (40 ml.) and methyl isobutyl ketone (40 ml.) was cooled to 5° C. With vigorous stirring, the mixture was adjusted to pH 0.9 and 5° C for 75 min. by the addition of further hydrochloric acid when necessary. The layers were separated and the aqueous layer washed with methyl isobutyl ketone (10 ml.) and residual solvent removed *in vacuo*. The aqueous solution was cooled to 5° C, adjusted to pH 7.5 with 10% sodium hydroxide solution and extracted with ethyl acetate (3 × 30 ml.). The combined organic extracts were dried over anhydrous magnesium sulphate and evaporated under reduced temperature and pressure to give the required penicillin ester.

EXAMPLE 3

Acetoxymethyl 6-[2,2-dimethyl-5-oxo-4-(p-hydroxyphenyl)-1-imidazolidinyl]penicillanate

(a) A suspension of 6- (−)-α-amino-*p*-hydroxyphenylacetamido]-penicillanic acid (5.0 g.) in acetone (40.0 ml.) was treated with triethylamine (3.6 ml.) and the mixture was stirred at room temperature for 24 hours. The supernatent solution was decanted from some insoluble gum and the solvent was distilled off *in vacuo*. The resulting glassy solid was dissolved in water (10.0 ml.) and the solution was adjusted to pH 2.5 by the addition of 5N hydrochloric acid. The resulting crystalline product was collected and dried *in vacuo* to give *6-[2,2-dimethyl-4-(p-hydroxyphenyl)-5-oxo-1-imidazolinyl]penicillanic acid* (2.2 g.), m.p. 198° (decomp.) (Found: C, 56.0; H, 5.8; N, 10.1. $C_{19}H_{23}N_3O_5S$ requires C, 56.3; H, 5.7; N, 10.4%).

(b) 6-[2,2-Dimethyl-5-oxo-4-(*p*-hydroxyphenyl)-1-imidazolidinyl] penicillanic acid (4.05 g. 0.01 mole, prepared as above, mixed with dry acetone (25 ml.) was treated with triethylamine (1.4 ml.). followed by bromomethyl acetate (1.53 g., 0.01 mole) and stirred at room temperature for 1 hr. The mixture was filtered through kieselguhr and the clear filtrate poured into ice water (150 ml.). The amorphous product which separated was collected, washed well with water and dried over phosphorous pentoxide *in vacuo* to give the required ester.

EXAMPLE 4

Pivaloyloxymethyl 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]-penicillanate hydrochloride

Crystalline pivaloyloxymethyl 6-aminopenicillanate p-toluene sulphonate (14.6 g 0.029 mol.) in ethyl acetate (707 ml.) was treated with a solution of 2% sodium bicarbonate (455 ml.) and well stirred. The organic layer was separated, washed with water, dried over anhydrous magnesium sulphate and evaporated *in vacuo* to give a residual oil.

Sodium (−)N-(1-methoxycarbonylpropen-2-yl) α-amino-α-(p-hydroxyphenyl) acetate (17 g. 0.059 mol.), prepared as in Example 1 (c) above, in ethyl acetate (236 ml.) was cooled with stirring to −15° C and treated with N-methylmorpholine (0.296 ml.) and ethyl chloroformate (5.66 ml. 1 equiv.). The mixture was stirred at −15° C for 6 minutes. An ice cold solution of the oily pivaloyloxymethyl 6-aminopenicillanate in ethyl acetate (118 ml.) was added to the mixed anhydride reaction mixture and the whole stirred at −12° to −14° C for 10 minutes followed by 1/2 hr. at room temperature. The reaction mixture was washed with 0.5 N sodium bicarbonate (59 ml.) followed by water (2 × 30 ml.). The organic layer was clarified by filtration through Kieselguhr, dried over anhydrous magnesium sulphate and evaporated *in vacuo* to give a yellow foam. The foam was dissolved in acetone (118 ml.) and water (106 ml.) and stirred while the pH was maintained at 2.5 by dropwise addition of 5N hydrochloric acid until no more acid was required (6.5 ml. of acid added during 20 minutes). The acetone was evaporated *in vacuo* and the residual aqueous phase extracted with ethyl acetate (118 ml.). The organic layer was diluted with 40—60° petroleum ether (94 ml.) and extracted with water at pH 3 (30 ml.). This aqueous extract, combined with the original aqueous solution, was treated with solid sodium chloride (28.4 g.) and stirred vigorously. An oily organic layer was separated and the remaining aqueous phase extracted with ethyl acetate (60 m.l). The combined organic phases were dried over anhydrous magnesium sulphate and evaporated *in vacuo*. The residue was dissolved in isopropanol (100 ml.), evaporated, and the residue redissolved in isopropanol (100 ml.). The clear solution was poured into dry ether (750 ml.) with stirring and the precipitated solid filtered, washed with dry ether and dried over phosphorous pentoxide *in vacuo* to give the product 13.37 g. (89.1%) as a colourless non-crystalline solid.

EXAMPLE 5

Pivaloyloxymethyl 6-[2,2-dimethyl-5-oxo-4-(p-hydroxyphenyl)-1-imidazolidinyl]penicillanate

6-[2,2-dimethyl-5-oxo-4-(p-hydroxyphenyl)-1-imidazolidinyl]penicillanic acid (16.20 g. 0.04 mol.), prepared as in Example 2 (a) above, in dimethylformamide (120 ml.) was treated with triethylamine (5.52 ml. 1 equiv.), stirred for 5 minutes to effect solution and bromomethyl pivalate (7.8 g. 1 equiv.) in dimethylformamide (20 ml.) was added. The solution was stirred at room temperature for 1 hr. A trace of solid was removed by filtration and the clear filtrate poured into ice water (600 ml.). The

separated solid was filtered, washed well with water and dried over phosphorus pentoxide *in vacuo* to give the product 7.7 g (37.1%) as a pale yellow non-crystalline solid.

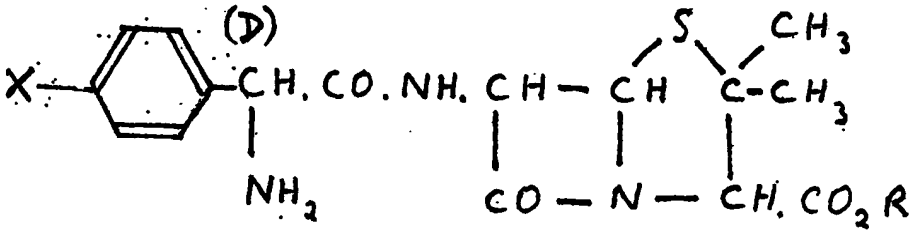
EXAMPLE 6

Pivaloyloxymethyl 6-[(−)-N-(1-methoxycarbonylpropen-2-yl)α-amino-α-(p-hydroxyphenyl)acetamido]penicillanate

Sodium 6-[(−)-N-(1-methoxycarbonylpropen-2-yl) α-amino-α-(p-hydroxyphenyl)acetamido] penicillanate (4.85 g. 0.01 mol.) in dimethylformamide (30 ml.) was treated with bromomethyl pivalate (1.95 g. 1 equiv.) in dimethylformamide (5 ml.) and stirred for 1 hr. at room temperature. The mixture was clarified by filtration and the filtrate was poured into ice water (150 ml.). The separated solid was filtered, washed well with water, and dried over phosphorus pentoxide *in vacuo* to give the product 4.03 g. (69.8%) as a colourless non-crystalline solid.

EXAMPLE 7

The following experiment shows that the concentrations of 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]penicillanic acid found in the serum of squirrel monkeys dosed by mouth with the corresponding pivaloyloxymethyl ester were greater than those which resulted from similar administration of equivalent quantities of the free acid. Each compound was given orally as a suspension to groups of monkeys in the fasting state, the dose being equivalent to 100 mg of free 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]penicillanic acid per kilogramme of body weight. Serum samples were taken at timed intervals and assayed for 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]penicillanic acid content against Sarcina lutea. Similar experiments were performed with ampicillin and its pivaloyloxymethyl ester, the blood levels in this case being expressed as concentrations of ampicillin. The four sets of average blood levels are shown in the table below:—



(III)

TABLE

Compound (III) administered		Concentration in μg/ml of aminopenicillin in serum				
X	R	½ hr.	1 hr.	2 hr.	4 hr.	6 hr.
OH	$CH_2OCOCCH_3$	22.2	25.4	20.7	6.8	2.2
OH	H	17.2	15.3	5.6	0.99	0.31
H	$CH_2OCOCCH_3$	11.9	9.0	3.2	0.63	0.15
H	H	6.5	9.4	3.1	0.45	0.07

EXAMPLE 8

(a) Benzoyloxymethyl 6-aminopenicillanate p-toluenesulphonate

6-Aminopenicillanic acid (27 g., 0.125 mol.) in dry acetone (17.3 ml.) was treated with triethylamine (17.3 ml.) and stirred for 1/2 hr. at room temperature. The solution was cooled to 0° C and bromomethyl benzoate (26.9 g., 0.125 mol.) in dry acetone

(54.5 ml.) added and the mixture stirred for 4 hours at 15—20° C. A further quantity of dry acetone (20 ml.) was added, the mixture poured into dry ether (625 ml.) and filtered. The clear filtrate was washed with saturated sodium bicarbonate (62.5 ml.) followed by saturated brine (62.5 ml.). The solution was treated with a solution of p-toluenesulphonic acid (15.9 g.) in dry acetone (250 ml.). After stirring for 1/2 hrs., the solid product was filtered, washed with dry ether and dried *in vacuo* to give the product 16.98 g. (25.9%) as a colourless crystalline solid M.P. 140—144° C (d). Recrystallisation from acetone/ether gave M.P. 139—143° C (d). Found: C, 52.21; H, 5.02; N, 5.31; S, 12.71. $C_{23}H_{25}O_8N_2S_2$ requires: C, 52.85; H, 5.02; N, 5.36; S, 12.28%, n.m.r. $(CD_3)_2SO$: δ=1.43 and 1.62 (6H.d. gemdimethyl), δ=2.30 (3H.s. p-methyl), δ=4.65 (1H.s.C_3 proton), δ=5.15 (1H.d. C_6 proton), δ=5.57 (1H.d. C_5 proton), δ=6.10 (2H.s.methylene), δ=7.15 and 7.58 (4H.q. p-substit aromatic), δ=7.78 (5H.m. benzoyl).

(b) Benzoyloxymethyl 6-[(−)α-amino-α-(p-hydroxyphenyl)-acetamido]penicillanate hydrochloride

Crystalline benzoyloxymethyl 6-aminopenicillanate p-toluene sulphonate (10.45 g., 0.02 mol.) was converted to the free base and reacted with the mixed anhydride obtained from sodium (−) N-(1-methoxycarbonyl-propen-2-yl)α-amino-α-(p-hydroxyphenyl)acetate(11.5 g., 0.04 mol.) as described in Example 4.

The final precipitated solid was filtered, washed with dry ether and dried *in vacuo* to give the product 5.74 g. (53.5%) as a colourless non-crystalline solid.

EXAMPLE 9

(a) Hexahydrobenzoyloxymethyl 6-aminopenicillanate p-toluenesulphonate

6-Aminopenicillanic acid (2i.6 g., 0.1 mol.) was reacted with bromomethyl hexahydrobenzoate b.p. 116—121° C/18/19 mm (22.1 g., 0.1 mol.) as described in Example 8 (a). The product 8.36 g. (15.8%) was isolated as a colourless crystalline solid m.p. 149—151° C. Found: C, 52.38; H, 6.16; N, 5.10; S, 12.63. $C_{23}H_{32}O_8N_2S_2$ requires: C, 52.26; H, 6.10; N, 5.30; S, 12.13%, n.m.r. $(CD_3)_2SO$ δ=2.30 (3H.s. p-methyl), δ=4.56 (1H.s. C_3 proton), δ=5.11 (1H.d. C_6 proton), δ=5.52 (1H.d. C_5 proton), δ=5.80 (2H.s. methylene), δ=7.11 and 7.53 (4H.q. p-substit. aromatic).

(b) Hexahydrobenzoyloxymethyl 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]-penicillanate hydrochloride

Crystalline hexahydrobenzoyloxymethyl 6-aminopenicillanate p-toluenesulphonate (8.99 g. 0.017 mol.) was converted to the free base and reacted with the mixed anhydride obtained from sodium (−) N-(1-methoxycarbonylpropen-2-yl)α-amino-α-(p-hydroxyphenyl)acetate (9.77 g., 0.034 mol.) as described in Example 4. The precipitated solid was filtered, washed with dry ether, and dried *in vacuo* to give the product 5.7 g. (61.8%) as a pale yellow non-crystalline solid.

EXAMPLE 10

(a) (3,5 - Dimethylisoxazole-4-carbonyl)oxymethyl 6-aminopenicillanate p-toluene sulphonate

6-Aminopenicillanic acid (5.92 g. 0.274 mol.) was reacted with bromomethyl 3,5-dimethylisoxazole-4-carboxylate (6.4 g., 0.0274 mol.) as described in Example 8 (a). The product 2.95 g. (19.9%) was isolated as a colourless crystalline solid m.p. 142—146° C (d). Found: C, 48.10; H, 4.97; N, 7.52; S, 12.04. $C_{22}H_{27}O_9N_3S_2$ requires: C, 48.78; H, 5.02; N, 7.76; S, 11.84%, n.m.r. $(CD_3)_2SO$ δ=1.45 and 1.65 (6H.d. gemdimethyl), δ=2.30 (3H.s. p-methyl, δ=2.35 (3H.s. 3-methyl), δ=2.63 (3H.s. 5-methyl), δ=4.62 (1H.s. C_3 proton), δ=5.14 (1H.d. C_6 proton), δ=5.56 (1H.d. C_5 proton), δ=6.01 (2H.s. methylene), δ=7.06, 7.20, 7.48 and 7.61 (4H.q. p-substit. aromatic).

(b) (3,5-Dimethylisoxazole-4-carbonyl)oxymethyl 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]penicillanate hydrochloride

Crystalline (3,5-dimethylisoxazole-4-carbonyl)oxymethyl 6-aminopenicillanate p-toluenesulphonate (2.71 g., 0.005 mol.) was converted to the free base and reacted with the mixed anhydride from sodium (−) N-(1-methoxycarbonylpropen-2-yl)α-amino-α-(p-hydroxyphenyl)acetate (2.87 g., 0.01 mol.) as described in Example 4. The precipitated solid was filtered, washed with dry ether and dried *in vacuo* to give the product 1.38 (49.7%) as a colourless non-crystalline solid.

EXAMPLE 11

Propionyloxymethyl 6[(−)α-amino-α(p-hydrovyphenyl)-acetamido]penicillanate hydrochloride could be prepared by the general procedure of Example 8, but starting with bromomethyl propionate instead of bromomethyl benzoate. Similarly isobutyryloxymethyl 6[(−)α-amino-α(p-hydroxyphenyl)-acetamido]-penicillanate hydrochloride could be prepared from bromomethyl isobutyrate, (2-ethylhexoyl)-oxymethyl 6[(−)α-amino-α(p-hydroxyphenyl)-acetamido]penicillanate hydrochloride could be prepared from bromomethyl 2-ethylhexoate, and phenylacetoxymethyl 6[(−)α-amino-α(p-hydroxyphenyl)-acetamido]penicillanate hydrochloride could be prepared from bromomethyl phenylacetate.

EXAMPLE 12

Pivaloyloxymethyl 6-[(−)α-amino-α(m-hydroxyphenyl)-acetamido]penicillanate hydrochloride could be prepared by the general procedure of Example 4, but using sodium (−)N-(1-methoxycarbonylpropen-2-yl)α-amino-α(m-hydroxyphenyl) acetate in place of the p-hydroxy isomer.

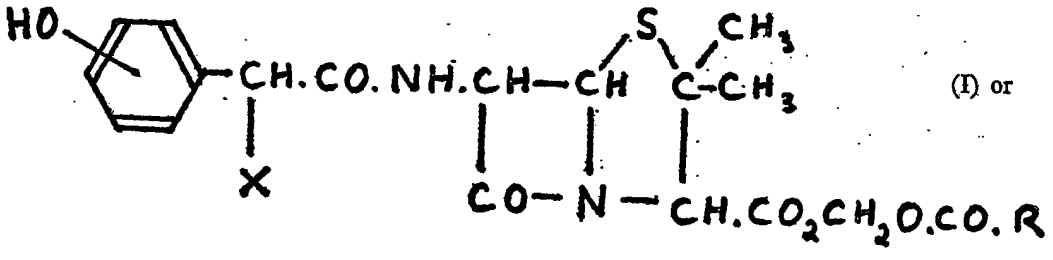
EXAMPLE 13

The average concentrations of 6[(−)α-amino-α(p-hydroxyphenyl(acetamido]penicillanic acid and of 6[(−)α-amino-phenylacetamido]penicillanic acid produced in the blood serum of two animal species following oral administration of the corresponding pivaloyloxymethyl esters were compared. Each ester was given by mouth to groups of rats (Sprague-Dawley CFY strain) or beagle dogs, the dose being equivalent to 100 mg. of the free amino penicillin per kilogramme of body weight. Serum samples were withdrawn at timed intervals after dosing, microbiological assay against Sarcina lutea giving the mean results tabulated below. In both species the p hydroxy compound generally gives rise to higher serum levels than its unsubstituted analogue, the difference being particularly marked some hours after dosing.

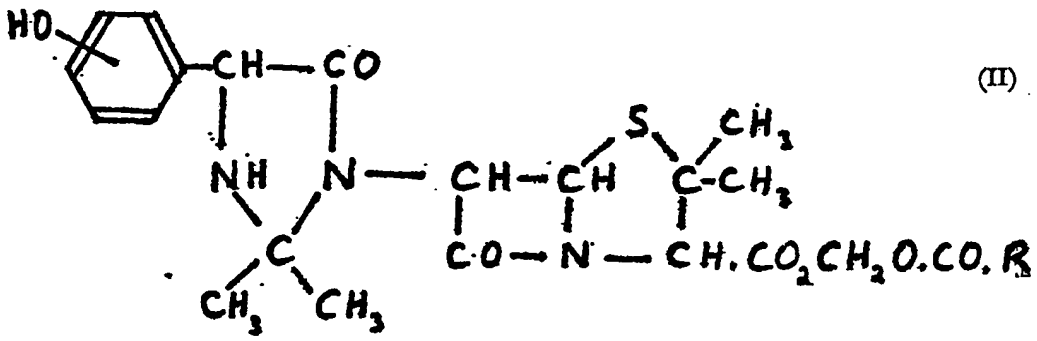
Animal species	Substituent in benzene ring	Concentration in μg/ml of aminopenicillin in serum				
		½ hr.	1 hr.	2 hr.	4 hr.	6 hr.
Rat	p Hydroxy	2.2	3.9	4.2.	1.9	1.37
Rat	None	4.3	3.9	1.9	1.04	0.65
Dog	p Hydroxy	6.1	8.6	8.4	3.4	1.9
Dog	None	4.0	3.9	1.4	0.31	0.06

WHAT WE CLAIM IS:—

1. A compound of the general formula:

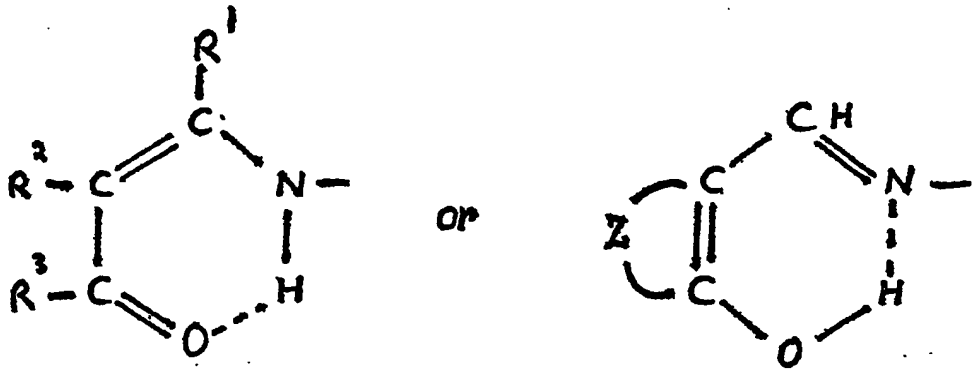


(I) or



(II)

where R is an alkyl, cycloalkyl, alkenyl, cycloalkenyl, aralkyl, aryl or heterocyclic group which may be substituted, and where in (I) X is NH_2, optionally in the form of an acid addition salt, or a protected amino group of the general formula:—



(III) (IV)

or tautomeric modifications thereof, wherein the dotted lines represent hydrogen bonds. R^1 is a lower alkyl group, R^2 is either H or together with R^1 completes a carbocyclic ring, R^3 is a lower alkyl or lower alkoxy group, and Z represents the residue of a substituted or unsubstituted benzene or naphthalene ring.

2. A compound according to claim 1 wherein the phenolic hydroxyl group is in the para position on the benzene ring.

3. A compound according to claim 1 wherein X is the 1-methoxycarbonylpropen-2-yl group.

4. A compound according to any one of claims 1 to 3 wherein R is lower alkyl having from 1 to 6 carbon atoms.

5. A compound according to any one of claims 1 to 4 wherein R is methyl or tertiary butyl.

6. A compound according to any one of claims 1 to 5 wherein X is NH_2.

7. Pivaloyloxymethyl 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]penicillanate or a non-toxic acid addition salt thereof.

8. Pivaloyloxymethyl 6-[2,2-dimethyl - 5 - oxo-4-(p-hydroxyphenyl)-1-imidazolidinyl]penicillanate or a non-toxic acid addition salt thereof.

9. Acetoxymethyl 6-[(−)α-amino-α-(p-hydroxyphenyl)acetamido]penicillanate or a non-toxic salt thereof.

10. Acetoxymethyl 6-[2,2-dimethyl-5-oxo-4-(p-hydroxyphenyl)-1-imidazolidinyl]-penicillanate or a non-toxic acid addition salt thereof.

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